PATENT COOPERATION TREATY

by fax and post

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

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PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	23.02.2001
Applicant's or agent's file reference: PCT-24409	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/08856	18/11/1999	19/11/1998

Applicant: S.I.S.S.A. SCUOLA INTERNATIONALE SUPERIORE..et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

   The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

   Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

   For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
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Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PCT-24409		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/08856	18/11/1999	19/11/1998
International Patent Classification (IPC) or national classification and IPC C12N15/10		
Applicant S.I.S.S.A. SCUOLA INTERNATIONALE SUPERIORE..et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
12/06/2000	23.02.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Kalsner, I Telephone No. +49 89 2399 8708

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/08856

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-54 as originally filed

Claims, No.:

1-92 as originally filed

Drawings, sheets:

1/8-8/8 as originally filed

Sequence listing part of the description, pages:

55-57, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	10-13, 17-23, 29-47, 49-73, 76-81, 90-92
	No:	Claims	1-9, 14-16, 24-28, 48, 74, 75, 82-89
Inventive step (IS)	Yes:	Claims	49-73, 92
	No:	Claims	10-13, 17-23, 29-47, 76-81, 90, 91
Industrial applicability (IA)	Yes:	Claims	1-92
	No:	Claims	

2. Citations and explanations
   see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Ad Section V: Reasoned statement with regard to novelty, inventive step or industrial applicability

1) Documents

D1...WO-A-97 20078
D2...Griffiths et al. (1994) EMBO J. 13: 3245-3260
D3...Tsurushita et al. (1996) Gene 172: 59-63

1.1) D1 describes the generation of nucleic acid libraries by in vivo recombination between two variant genes present in different plasmids. The method disclosed comprises the combination of at least two members of an initial population of nucleic acid molecules in a recombination system resulting in recombination of at least two members of the population, thereby producing a population comprising recombined nucleic acid members. The recombination can be homologous recombination or site-directed recombination, e.g. via cre/lox or flp/frt systems (p. 6/7; p. 25).

1.2) D2 discloses a method for the generation of antibody libraries by in vivo recombination of a donor heavy chain library (on a plasmid) and an acceptor light chain library (on phage). The two chains are combined on the same replicon and recombination is catalysed via Cre recombinase and the loxP/loxP511 sites.

1.3 D3 discloses the generation of large combinatorial libraries by in vivo recombination of immunoglobulin heavy and light chain genes using the Cre/loxP system. A plasmid is disclosed where the loxP sequence is comprised in the polypeptide link between the antibody V regions to form a scFv (p. 60, right col., lines 18-20).

2) Novelty

2.1) **Claim 1**, which broadly defines a method of preparing a nucleic acid library, dependent **claims 2-9, 14-16 and 24-28** which further define the method of claim 1 and **claims 74 and 75** which refer to a method of preparing a polypeptide employing a nucleic acid library according to the method of claim 1, cannot be

considered novel in view of the disclosure of D1 (Art. 33(2) PCT).

2.2) **Claim 48** does not meet the requirements of Art. 33(2) PCT as nucleic acid libraries are known in the state of the art (see, e.g. D1, D2, D3). It should be noted that known products are not rendered novel by producing them by a possibly new process.

The same argument holds true for **claims 82-84** which refers to a polypeptide and a host cell.

2.3) **Claims 85-89** relating to a vector encoding a single chain antibody are not considered novel in view of D2 and D3.

3) Inventive step

3.1) **Claims 10-13** refer to the method of claim 1 further characterising the recombinase recognition sites. Using a loxP and loxP mutant site in the construction of a combinatorial library, however, is known in the state of the art (e.g. D3). Claims 10-13, thus are not considered to involve an inventive step (Art. 33(3) PCT).

3.2) **Claims 31-43** do not meet the requirements of Art. 33(3) PCT as the subject-matter of these claims is not considered to involve an inventive step in view of D1 in combination with D2.

3.3) **Claims 17-23, 29, 30, 44-47 and 76-81** do not meet the requirements of Art. 33(3) PCT as they do not add any features which would render inventive the subject-matter of the claims they refer to.

3.4) **Claims 90 and 91** do not meet the requirements of Art. 33(3) PCT as a kit comprising a container and a known substance is not considered to involve an inventive step.

3.5) **Claims 49-73 and 92** are considered to meet the requirements of Art. 33(2)(3) PCT as a nucleic acid library wherein every member of said library has the same

origin of replication is not disclosed or suggested in the available prior art.

Ad Section VIII: Certain observations on the international application

The present set of claims does not meet the requirements of Art. 6 PCT for the following reasons:

1) The present application relates to the generation of nucleic acid libraries containing multiple variants of two parent nucleic acid molecules by in vivo recombination of two phagemid vectors containing the same origine of replication and variants of a sequence which are flanked by different recombinase recognition sites. It appears that an essential feature of the invention is the fact that recombination occurs between two constructs which have the same origin of replication.

   This feature, however, is not reflected in the majority of the claims, thus rendering the claims unclear.

2) **Claims 38, 39 and 41** do not meet the requirements of Art. 6 PCT as the dependencies of these claims are not clear:

   Claim 38 refers to the method of claim 36 "wherein said loxP sites are selected....". Claim 36, however, does not mention any loxP sites.

   Claims 39 and 41 refer back to claim 29 making reference to "said library". Neither claim 29 nor claim 26 which claim 29 depends on specify a library.

3) **Claim 48** does not meet the requirements of Art. 6 PCT, as it is not defined by technical features. The claim is considered unclear as it does not enable the skilled person to unambiguously determine whether a given nucleic acid library would fall under the scope of the claim or not.

4) **Claim 80** does not meet the requirements of Art. 6 PCT for the following reasons:

   Claim 80 refers to the method of claim 75 which depends on claim 74 and thus

indirectly refers to the method of claim 1. It is not clear how the product of the third step of the method of claim 75 (which is produced by a method of which the method of claim 1 is a prerequisite) can serve to generate a library according to the method by which it is created.

5) **Claims 82 and 83** do not meet the requirements of Art. 6 PCT as the polypeptide and the host cell, respectively, are not defined by any technical features. The skilled person is thus not in the position to determine without undue burden whether or not a given polypeptide or host cell falls within the scope of the claims.